368-Pos Board B168
Novel Polarizable Empirical Code for Accurate Prediction of Protein-DNA Interactions
Carlos J. Camacho.
Traditional molecular dynamics techniques can compute free energy differences between well defined states, but currently they cannot consistently estimate the full contribution of the solvent. In parallel, considerable efforts are being devoted to the development of polarizable force fields. Despite their solid theoretical foundation, these methods have yet to reach their full potential and, equally important, justify their computational cost. On the other hand, there is an urgent need to improve the predictive power of physically based empirical potentials to better understand the molecular basis of protein interactions and function, and expedite molecular engineering and drug design. Here, we propose a comprehensive and scalable approach to include polarization/dielectric effects that uses a novel structure based method to estimate physical interactions in proteins. The main hypothesis is that varying dielectric environments can be approximated by a ''water factor'' derived from a structural analysis of molecular water at the binding interface. Our approach goes beyond solvent accessible surface area techniques: both the accessibility to solvent and the nature of the water network (''bulk'', ''trapped'', or ''crystal'') are taken into account. Applications to DNA interactions of C2H2 zinc fingers transcription have shown that such a water factor improves the R2 correlations between predicted and experimental changes in binding free energy from around 0.6 to better than 0.94.

369-Pos Board B169
Probing the Conformational Dynamics of Protein-DNA Complexes Through Hydration
Gregory M.K. Poon.
The DNA-binding domain of the ETS family of transcription factors interacts with DNA in a sequence-selective manner, yet it tolerates remarkable variation in the recognition sequence. The biophysical basis for this sequence selectivity is currently unknown. Previous investigations have found significant differences in the thermodynamics of strong and weak ETS-DNA complexes. However, the ETS domain is structurally similar in both DNA-bound *and* unbound states. In the absence of significant coupled folding by either protein or DNA, selectivity may be linked to differences in conformational dynamics in the protein-DNA complex. Specifically, sequence selectivity may arise from differential reductions in conformational dynamics upon complex formation. We hypothesize that time-averaged differences in conformation dynamics can be resolved through their equilibrium hydration properties. The rationale for our hypothesis is that folded but conformationally mobile segments should be more solvent-accessible than segments that are relatively immobilized. To test this hypothesis, we are probing the hydration of various ETS-DNA complexes by high-resolution DNA and protein footprinting. We expect that this approach will provide a quantitative measure of a complex's overall conformational dynamics as well as identify structural elements that become conformationally stabilized upon complex formation.

370-Pos Board B170
Target Site Localization Dynamics of DNA-Binding Proteins in Vivo
Elena F. Koslover, Mario A. Diaz de la Rose, Stephanie C. Weber, Andrew J. Spakowitz.
Genetic regulatory responses are limited by the dynamics of transcription-factors searching for specific binding sites on DNA. This target search process is thought to occur by facilitated diffusion, a combination of three-dimensional diffusion and one-dimensional sliding. While facilitated diffusion is capable of significantly speeding up the search in vitro, the importance of this process in vivo remains unclear. Furthermore, the scaling of transport processes within the cell is modified by crowding and biological fluctuations, which thereby play a role in modulating the speed of regulatory response. We present a set of simulations for modelling the target-search dynamics of DNA-binding proteins in conditions relevant to both in vitro and in vivo settings. The simulations are used to address the role of DNA concentration and packing, as well as the effect of subdiffusive transport in the viscoelastic medium of the cytoplasm. We develop an effective theory for localization time-scales of DNA-binding proteins in vivo and extend our results to study the dynamics of regulatory transcription cycles in the cell.

371-Pos Board B171
Measurements of Force-Driven Changes in Bound Protein Numbers on a Single DNA
Botao Xiao, Houyin Zhang, Reid C. Johnson, John F. Marko.
DNA compaction and chromosome organization involve the dynamic interaction of long DNA molecules and many copies of various proteins. Determining numbers of proteins bound to large DNAs is important to understand their chromosomal functions and protein numbers may be affected not only by chemical factors, but also by physical factors such as mechanical forces generated in DNA, *e.g.*, by transcription or replication [(1)]. We performed single-DNA stretching experiments with bacterial nucleoid proteins HU [(2)] and Fis, where we verified that the force-extension measurements were in thermodynamic (chemical-mechanical) equilibrium. Given thermal equilibrium of protein binding, we could use a thermodynamic Maxwell relation to deduce the change of protein number on a single stretched DNA due to varied applied force. For the binding of both HU and Fis under conditions where they compact DNA, the numbers of bound proteins decreased as force was increased from 0.03 to 12 pN. This effect saturated with force for HU, but did not for Fis, reflecting the tighter binding of the latter to DNA. The experimental results agree well with expectations of binding numbers based on electrophoretic mobility shift assay data, and the HU data agree well with results from a simple statistical-mechanical model of DNA-bending proteins. This thermodynamic approach may be applied to measure force-driven changes in numbers of a wide variety of molecules bound to DNA or to other polymers; in the case of proteins binding to DNA, force-dependent binding suggests mechano-chemical mechanisms for gene regulation.
1. C. Bustamante, Z. Bryant, S. B. Smith, *Nature,* (2003).
2. B. Xiao, R. C. Johnson, J. F. Marko, *Nucleic Acids Res*, (2010).

372-Pos Board B172
Sequence Dependence of Binding and Exchange of Nonspecific Dna-Binding Proteins
John S. Graham, Reid C. Johnson, John F. Marko.
The multistep kinetics through which DNA-binding proteins bind their targets are heavily studied, but relatively little attention has been paid to mechanisms of how proteins leave the double helix. Using single-DNA stretching and fluorescence detection, we recently demonstrated that the sequence-neutral DNA-binding proteins HU, NHP6A and Fis, readily exchange with each other and that the rate and degree of exchange is dependent on the concentration of solution-phase protein, regardless of protein species. We now examine the sequence dependence of binding and the exchange reactions investigated previously. Our results indicate an apparent disparity between biochemically measured sequence dependence and the sequence dependence in our single molecule approach. Additionally, we demonstrate a coarse-grained sequence dependence of the exchange reactions and correlate those results with our observed binding specificity.

373-Pos Board B173
Stretching DNA to Quantify Non-Specific Binding by the Lambda Repressor (ci)
Sachin Goyal, Chandler Fountain, David Dunlap, Fereydoon Family, Laura Finzi.
Non-specific binding of regulatory proteins to DNA can be an important mechanism for target search and for storage. This seems to be the case for the λ repressor protein (CI), which maintains lysogeny after bacteriophage infection. CI binds specifically at two distant regions along the viral genome and induces the formation of a repressive DNA loop. However, single-molecule experiments and kinetic measurements show that CI also binds to DNA non-specifically, and that this mode of binding may play an important role in maintaining lysogeny. Therefore, we have quantified non-specific CI binding by stretching DNA at various CI concentrations using magnetic tweezers. We recorded the decrease in DNA extension caused by CI (see Fig. 1). Then, we used a novel theory extending work by Zhang & Marko [PRE 77, 031916, (2008)] to calculate the change in CI binding from the measured decrease in DNA extension. In this presentation, we will interpret new stretching data at various CI concentrations using our novel theory. Furthermore, we will discuss the versatility of our method for characterizing gene regulation via non-specific protein binding.

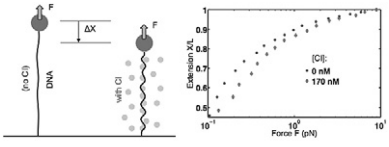

Fig. 1: (Left) A cartoon showing the decrease in DNA extension due to non-specific binding of CI proteins (green circles). (Right) DNA extension vs. force measured using magnetic tweezers in the absence (blue dots) and presence (red diamonds) of CI proteins [Data reported in JPCM (2010)].

374-Pos Board B174
FRET and Sm-FRET Characterization of a Lac Repressor-Dna Looping Landscape
Jason D. Kahn, Aaron R. Haeusler, Kathy A. Goodson, Douglas S. English.
DNA looping is fundamental to transcriptional regulation, and the archetypal loop is formed by the Lac repressor protein, LacI. Thermodynamic characterization of looping energetics with and without induction is a necessary input for quantitative modeling of gene expression, and the LacI system is also a test case for statistical mechanics and rod mechanics models of protein/DNA flexibility. We have systematically constructed a landscape of LacI-DNA looping variants patterned on previously developed molecules in which lac operators bracket a sequence-directed A-tract bend. The hyperstability of these loops enables study of LacI protein geometries that would otherwise be unstable with respect to unlooped single- or double-bound DNA. FRET donors and acceptors (Alexa